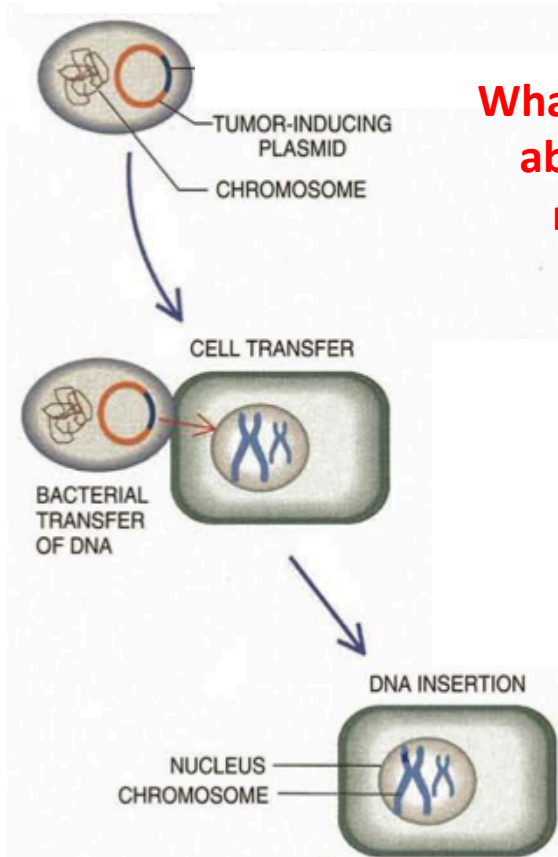# Introduction to Genotyping

HC70AL
Summer 2014
8/7/14

By

Michael Lyons

How were the *Arabidopsis thaliana* plants mutated?

What organism did we learn about in HC70A that is a natural plant genetic engineer?

How does Agrobacterium manipulate the plant genome?

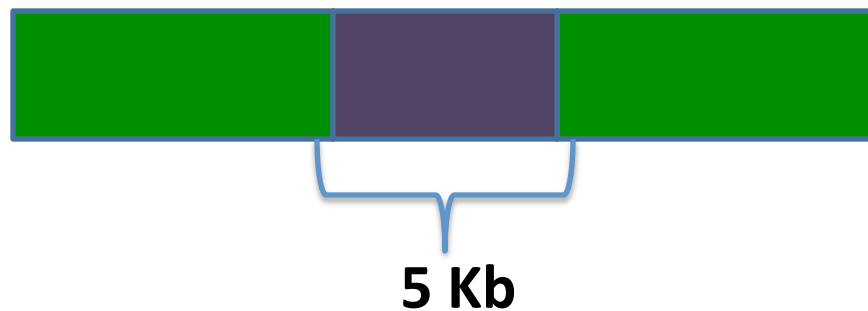**Where did the T-DNA get inserted?**

Gene of interest

T-DNA insert Within gene

What are the two possible alleles present in your plants?
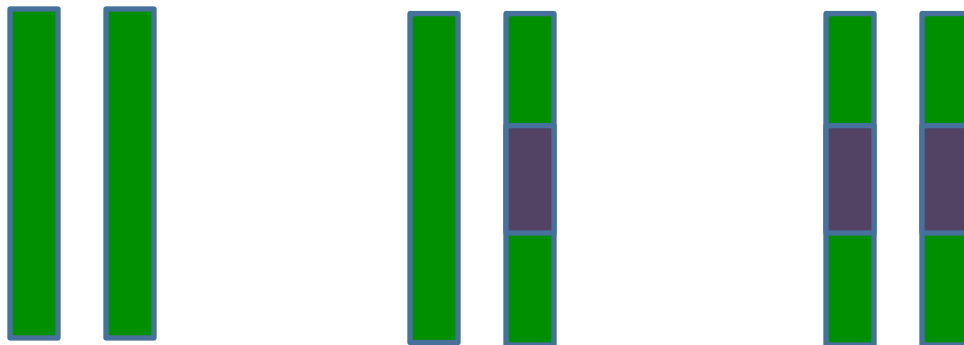
- Wild type allele of the gene

- Gene with T-DNA insert

5 Kb

What are the possible genotypes?
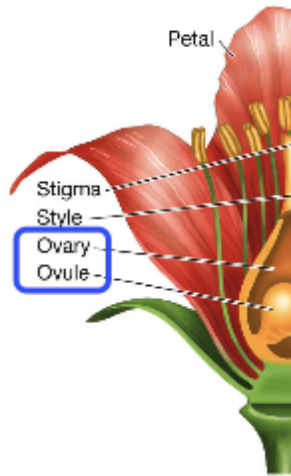
Homozygous Wild Type   Heterozygous   Homozygous for T-DNA
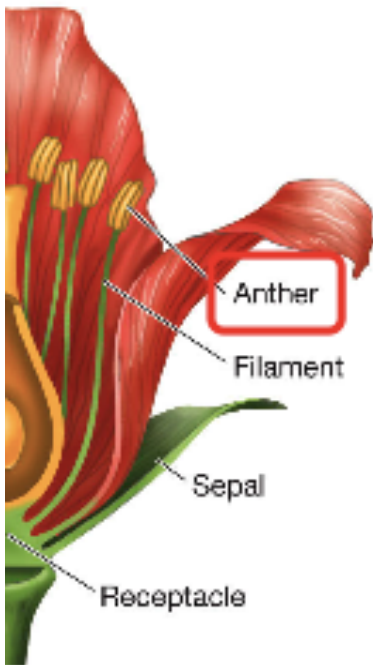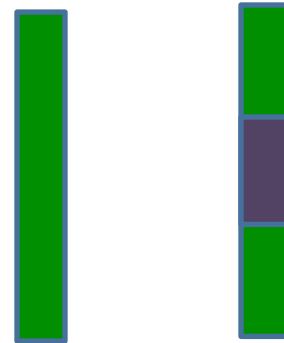
How can the different genotypes affect the phenotype of the plant?

What is the expected ratio of genotypes?

Where are the male gametes coming from? What about the female gametes?
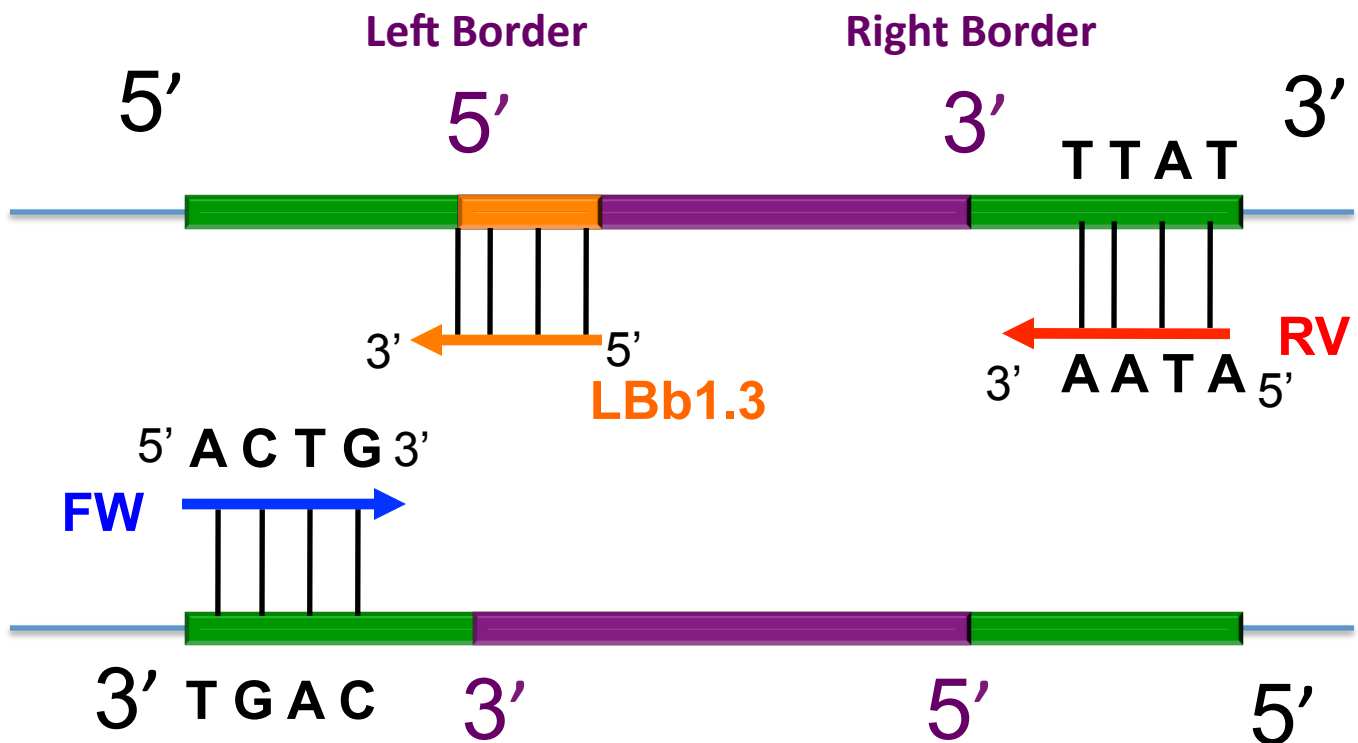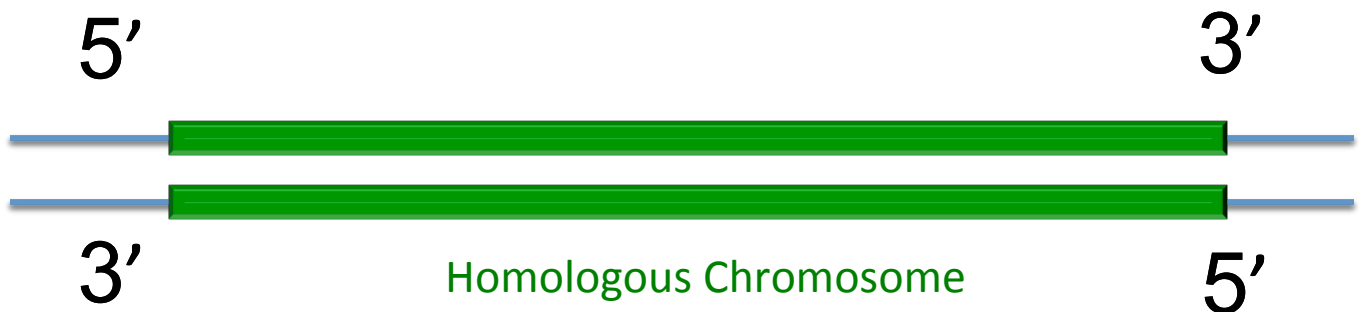

Petal
Stigma
Style
Ovary
Ovule

Female Gametes

Anther
Filament
Sepal
Receptacle

Male Gametes

	Wild Type	Hetero
Hetero		**Homo T-DNA**

How can we test if there is a T-DNA insert in a chromosome?

Where do the gene specific SALK forward and reverse primers bind? What about the LBb1.3 primer?

Left Border

Right Border

5'   5'   3'   3'

T T A T

3' ←   5'

RV

LBb1.3

3' A A T A 5'

5' A C T G 3'

FW →

3' T G A C   3'   5'   5'

What primer does the LBb1.3 primer form a PCR Product with?

5'   3'

3'   5'
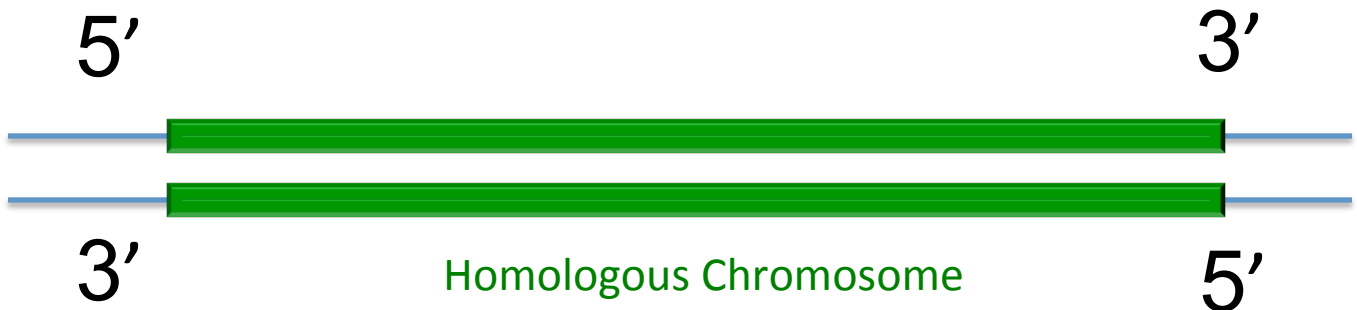
Homologous Chromosome

Will you get a PCR product using the LBb 1.3 primer if there is no T-DNA insert in the chromosome?

What would be our product if our T-DNA insert had the opposite orientation?

Does the T-DNA have to insert itself into the plant in a specific orientation?

5'   5'   3'   **T T A T** 3'

RV

3' **A A T A** 5'

5' **A C T G** 3'   **LBb1.3**

FW   5'   3'

3' **T G A C** 3'   5'   5'

Right Border   Left Border

What primer does the LBb1.3 primer form a PCR Product with?

5'   3'

3'   Homologous Chromosome   5'
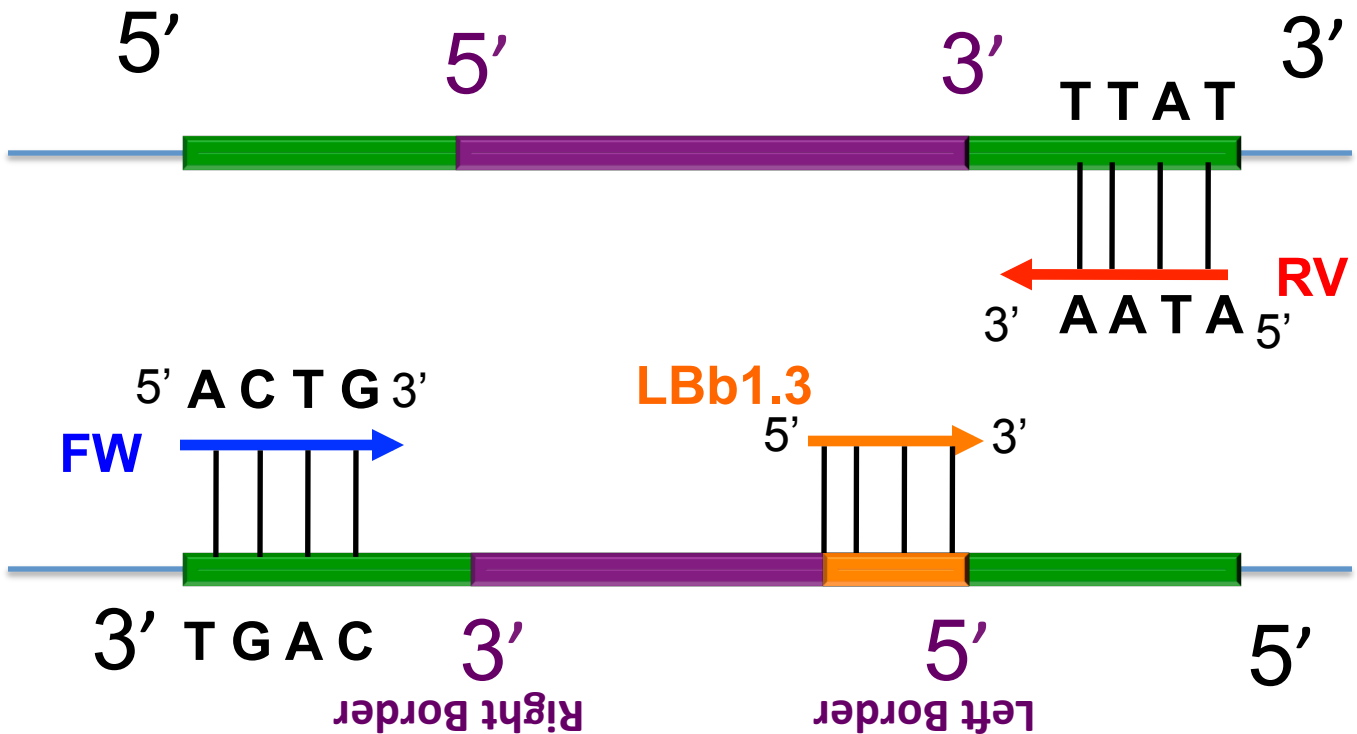
How many different sets of primers are we going to test to determine the genotype of our plants?

How do you set up a PCR reaction for genotyping?

What is different between each set of reactions? What is the same?

MASTER MIX A	MASTER MIX B	MASTER MIX C

Gene Specific SALK FW + LBb1.3

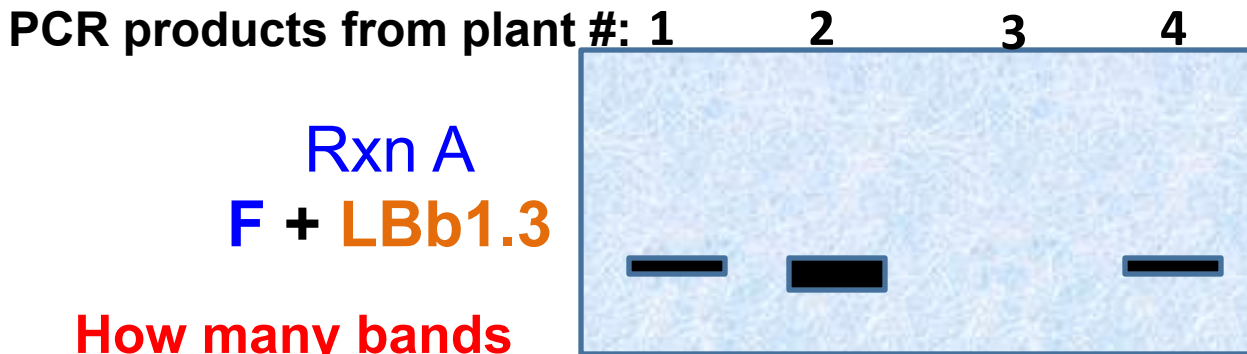**Gene Specific SALK RV + LBb1.3**

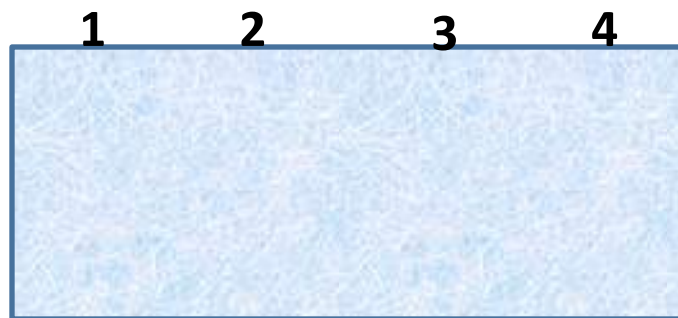**Gene Specific SALK FW + Gene Specific SLAK RV**

How many PCR reactions should you prepare with your master mix for if you have 6 plants, a positive control, and a negative control?

How do we interpret the gel results?

How many alleles does each plant have?

PCR products from plant #: 1   2   3   4

Rxn A
F + **LBb1.3**

How many bands would you expect to see in a homozygous plant?

Rxn B
R + **LBb1.3**

How many bands would you expect to see in a heterozygous plant?

Rxn C
F + **R**